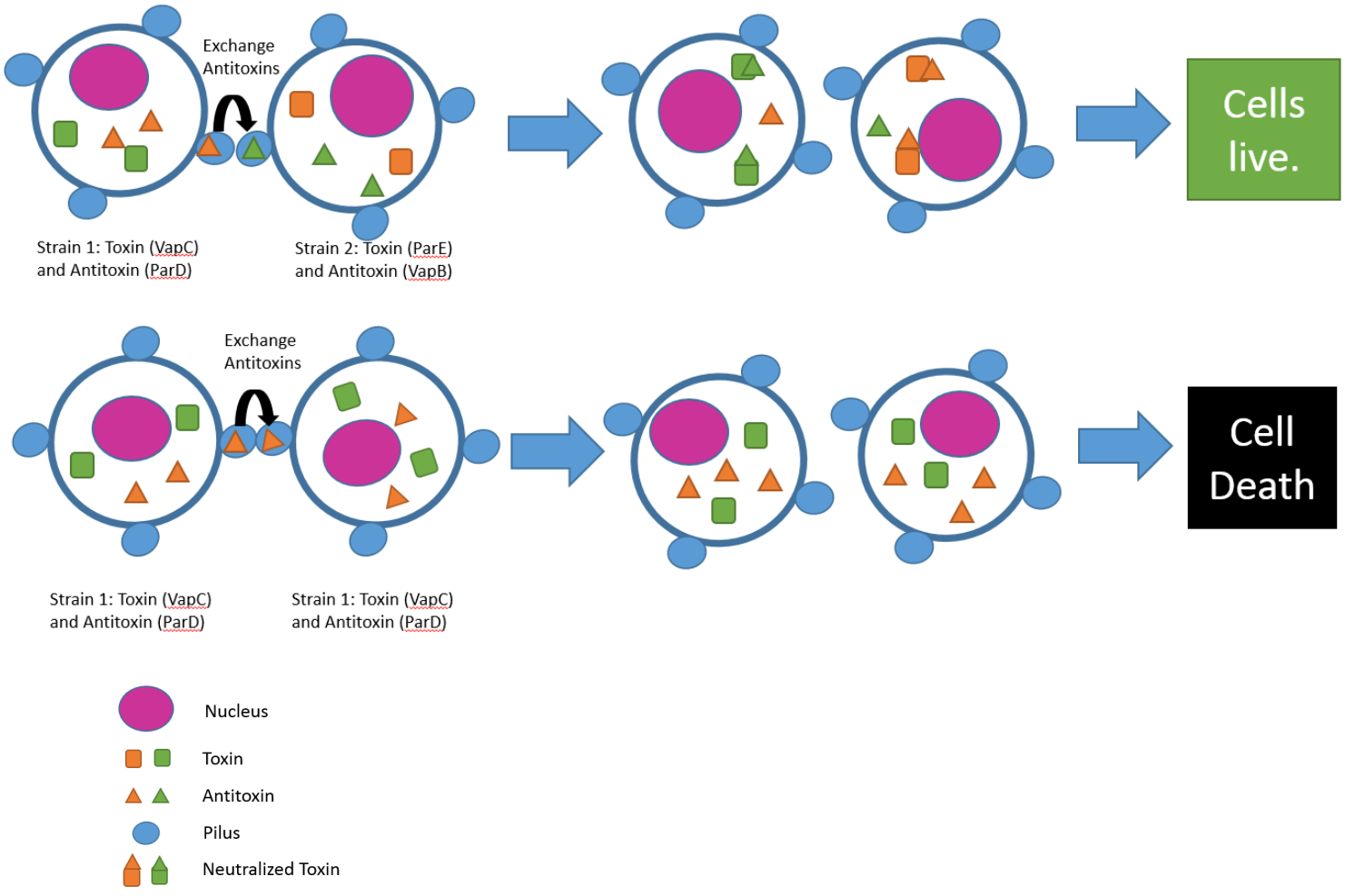
One alternative to a kill-switch proposed above involves two different *E. coli* strains with two different toxin-antitoxin (TA) systems in which the anti-toxins are swapped between the two strains. Both strains would have a pilus-based secretion system so that they can swap either toxins or anti-toxins despite the flowing water (Hayes et al., 2010). This way if one *E. coli* were to escape its toxin would have no anti-toxin to counteract it. This would be self-regulating for if one strain of *E. coli* became too numerous the several *E.coli* would die until a balanced population is restored. To ensure that the kill-switch swiftly eliminates the bacteria that escape, and produces the anti-toxin at a fast enough rate to prevent unnecessary death of the bacteria that have remained in the gel, all of the toxins and anti-toxins would be constitutively expressed

The TA systems are proposed to use are the VapC/B and ParE/D. VapC/B is normally found in *Mycobacterium tuberculosis*, and ParE/D is found in *Caulobacter crescentus*. These two were chosen because both systems have been shown to be toxic to *E. coli* and because the bacteria these TA systems are naturally occur in are found in lakes (Jiang et al., 2002; Zhang et al., 2004). They were chosen in an attempt to reduce any unforeseen impact a new toxic could have on an ecosystem. Keeping this desire in mind, a new possibility of the *E. coli* strains receive the anti-toxin from another bacteria is possible. Therefore the secretion system that will be used will be one that has little homology to ones found in other bacteria or archaea that are naturally found in a lake so as to reduce the risk of transferring those toxins to those naturally occurring species.



Pro: The kill-switch does not depend on environmental conditions which therefore allows it to be used in any body of water, at any depth, or during any time of day. By using toxin and anti-toxins already present in the ecosystem, this prevents some unforeseen outcomes that introducing new species or proteins into an environment can have.

Con: Since the current encapsulation plan does not include providing an energy source for the bacteria, this kill-switch could significantly reduce the viability of the cells as this process will divert energy away from the cells to bioremediate the mercury. Since toxin and anti-toxin can be found in ecosystem, it is possible that the bacteria could receive the correct anti-toxin that way.

Hayes, C.S., Aoki, S.K., and Low, D.A. (2010). Bacterial contact-dependent delivery systems. Annu. Rev. Genet. *44*, 71–90.

Jiang, Y., Pogliano, J., Helinski, D.R., and Konieczny, I. (2002). ParE toxin encoded by the broad-host-range plasmid RK2 is an inhibitor of Escherichia coli gyrase. Mol. Microbiol. *44*, 971–979.

Zhang, Y.X., Li, J., Guo, X.K., Wu, C., Bi, B., Ren, S.X., Wu, C.F., and Zhao, G.P. (2004). Characterization of a novel toxin-antitoxin module, VapBC, encoded by Leptospira interrogans chromosome. Cell Res. *14*, 208–216.